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Fast and single solid phase fluorescence spectroscopic batch procedure for (acetyl) salicylic acid determination in drug formulations

S. Ortega Algar, N. Ramos Martos, A. Molina Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje Las Lagunillas, E-23071 Jaén, Spain

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Abstract

A solid phase fluorescence spectroscopic batch procedure for (acetyl) salicylic acid in drug formulations have been developed. The procedure is based on the sorption of salicylic acid (SA) on Sephadex DEAE A-25 anion exchanger gel (100 mg) by equilibration from an aqueous solution (10 or 25 ml) for 5 min; the equilibrated gel is transferred into an 1 mm quartz cell and the native fluorescence of SA sorbed on it is directly measured ($\lambda_{ex} = 297$ nm; $\lambda_{em} = 405$ nm). Good linearity was found in the 10–200 and 5–100 μg l^{-1} ranges (for 10 and 25 ml sample volume, respectively) with R.S.D. (%) of 2.8 and 1.1. The procedure was successfully applied to the determination of acetyl salicylic acid (ASA) in drug formulations after alkaline hydrolysis to yield SA.

Keywords: (Acetyl) salicylic acid; Solid-phase fluorimetry; Drug formulations

1. Introduction

A variety of analytical techniques (spectrophotometry [1,2], spectrofluorimetry [3–5], chromatography [6–8]) have been used and a large number of methods published for the determination of acetyl salicylic acid (ASA) and salicylic acid (SA) in both biological fluids and pharmaceuticals. This is indicative of both the great interest in the determination of these two compounds and the problems encountered with their determination with respect to simplicity, selectivity, sensitivity, rapidity, etc.

Spectrofluorimetry seems to be the most suitable analytical technique due to its simplicity and sensitivity. Nevertheless, it often requires sample

* Corresponding author. Tel.: +34-53-012147; Fax: +34-953-012-141.
E-mail address: amolina@ujaen.es (A. Molina Díaz).

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pre-treatment procedures in order to avoid interference from other compounds. SA shows an intense fluorescence and several trace analysis methods are based on its native fluorescence [3,9,10]; ASA can also be determined directly by use of its native fluorescence, but more commonly is measured after hydrolysis to SA [8,9,11,12].

Solid surface luminescence [13] (the analytical measurement of the fluorescence or phosphorescence of a component sorbed on solid materials) has been used for enhancing the luminescence signal of a component (or its reaction product with a derivative reagent). Several solid supports have been used such us filter paper [14], silica gel [15], ion exchange resins [16], glasses prepared from sugars [17], non ion exchangeable gels [18].

Polymeric solid substrates have been used in recent years for concentrating fluorophors substances [19]. When the sorbent is an ion-exchanger, in addition to the enhancement of sensitivity due to the concentration of the fluorophor species on it, a drastical improvement in selectivity is also achieved because all ionic species with different charge from that the interest are excluded from the solid support. So, very selective and sensitive fluorimetric procedures have been published by using the batch mode by means of equilibrating a known amount of an ion-exchanger resin with a definite volume of sample solution [20,21]. The solid phase beads are collected and transferred to an appropriate quartz cell (usually of 1 mm optical path length) and the fluorescence of the fluorophor on the solid surface is directly measured. Both inorganic and organic analytes have been determined by Solid Phase Spectrofluorimetry [16,18–23]. This principle has also been used in automatic mode, combined with a non-segmented continuous flow system (FIA) [24].

Nevertheless, the batch mode has the advantage of using a much more single instrumentation, so providing extraordinarily inexpensive procedures when a native property is used as analytical signal [25]. However, batch mode has been scarcely applied to the determination of active principles in pharmaceuticals [26–28].

In this paper we propose a rapid straightforward solid-phase spectrofluorimetric procedure for the determination of SA based on its retention on Sephadex DEAE A-25 anion exchanger gel and the direct measurement of its native fluorescence sorbed on the solid support. The method can also be used for the indirect determination of ASA previous alkaline hydrolysis to SA.

The procedure shows a detection limit of 1.1 μg l^{-1} and does not require any pre-treatment of the sample other than its dissolution. Other accompanying species such as codeine and caffeine do not interfere at the usual concentrations, so no separation is required. The method is fast, simple and more inexpensive and more sensitive than other published method in the literature [1–10,29].

2. Experimental

2.1. Apparatus

Fluorescence measurements were obtained with a Perkin–Elmer LS-50 spectrofluorimeter (Beaconsfield, Buckinghamshire, UK) equipped with a xenon discharge lamp (20 kW), Monk-Gillieson monochromators, a Quantic Rhodamine 101 counter to correct the excitation spectra and a gated photomultiplier. The luminescence spectrometer was interfaced with a Mitac MPC 3000F-386 microcomputer supplied with FL DATA MANAGER Software v. 2.50 for spectral acquisition. Instrument excitation and emission slits were 2.5 and 5 nm, respectively, and the scans were obtained at 240 nm min^{-1}. The computer was also connected to an Epson LX-800 printer for delivery of results.

All measurements were made with a quartz cell of 1 mm path length at 20 °C, the temperature was controlled to within ±0.1 °C with aid of a Selecta (Barcelona, Spain) Frigiterm 6000-382 ultrathermostat.

The pH measurements were made with a Crison 2000 digital pH-meter (Barcelona, Spain) furnished with a combined glass/saturated calomel electrode. The meter was calibrated with two buffers at pH 4.0 and 7.0.

An Ultrasons Selecta (Barcelona, Spain) ultrasonic bath and an Agitaser 2000 (Barcelona, Spain) rotating agitator were also used.

2.2. Reagents

Stock solutions containing 10^2 mg l^{-1} of SA (Fluka, Madrid, Spain) were prepared by dissolution of the appropriate amount in deionised water. This solution is stable for 2 weeks stored in a refrigerator at 5 °C. Working solutions were daily prepared by suitable dilution.

A KH_2PO_4/NaOH (Merck, Darmstadt, Germany) buffer solution pH 7.0 at $c_T = 0.1$ M was also used.

Sephadex DEAE A-25 cation-exchange gel (Aldrich, Alcobendas, Madrid, Spain) was used in the H^+ form as the solid support without any pretreatment.

2.3. Fluorescence measurements

The measured signal of the fluorescent analyte fixed on the solid phase that is packed in a 1 mm silica cell was the diffuse transmitted fluorescence emitted from the resin at the unirradiated face of the cell. The optimum angle between the cell plane and the excitation beam was 45° [30].

2.4. Treatment of samples

2.4.1. Tablets

Twenty tablets were crushed to a fine powder and dissolved in water by sonication.

2.4.2. Capsules

The contents of 20 capsules were carefully transferred and dissolved by sonication.The solutions was filtered through a 0.45 μm pore size Millipore filter, and diluted to an appropriate volume with deionised water. The ASA determination requires its previous alkaline hydrolysis, by means of a sample treatment with 1 M NaOH solution, by heating 30 min at 60 °C. It was performed in those pharmaceutical containing ASA in order to obtain complete transformation to SA. Suitable dilutions were performed in all cases.

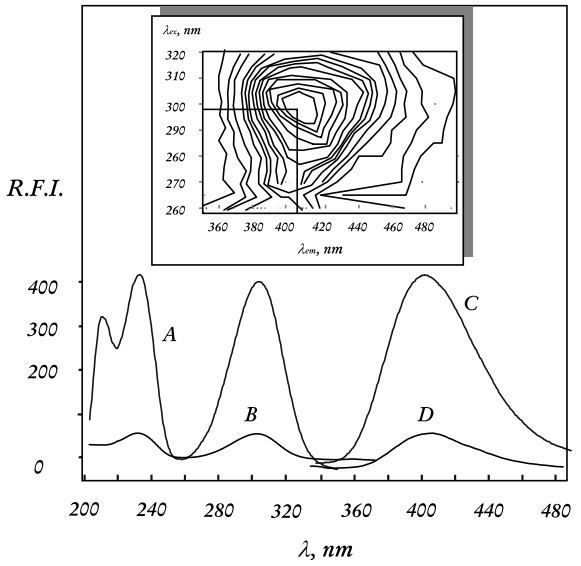

Fig. 1. Fluorescence spectra (excitation and emission spectra) of SA, (A, C) equilibrated on Sephadex DEAE A-25 resin; (B, D) in aqueous solution. SA 150 μg l^{-1} (sample volume, 10 ml). Inset, contour plot.

2.5. Procedure

2.5.1. Method for 10 ml of sample

A 10 ml sample volume containing an analyte concentration between 10 and 200 μg l^{-1} and 1 ml of buffer solution (pH 7.0) were transferred into a 25 ml test tube with stopper and 100 mg of Sephadex DEAE A-25 resin were added. These tubes were shaken mechanically for 5 min after which, the solid phase was collected from the bottom of the test tube and packed into a 1 mm quartz cell together with a small volume of the equilibrated solution. To improve the resin packing the cell was centrifuged for 2 min at 2000 rpm. The relative fluorescence intensity at 20.0 ± 0.1 °C was measured at $\lambda_{em} = 405$ nm (5 nm slit-width) using $\lambda_{ex} = 297$ nm (2.5 nm slit-width).

Every concentration used to construct the calibration line (concentration range 10–200 μg l^{-1}), and the blank solution were prepared, and also treated in the same way as described for the sample.

2.5.2. Method for 25 ml of sample

Operate similarly but using 2.5 ml of buffer solution and a calibration range from 5 to 100 μg l^{-1}. Fifty millilitres polyethylene test tubes were used for equilibration.

3. Results and discussion

3.1. Spectral characteristics

Due to dissociation, SA can be sorbed on an anion-exchanger gel as salicylate anion. So, from a definite sample volume, it can be concentrated on a small amount of exchanger gel. When the beads of the gel are transferred into a quartz cell of 1 mm optical path length, a strong increase in the native fluorescence signal of SA can be observed compared with that in aqueous only solution, measured in the same cell (without solid support). Fig. 1 shows the excitation and emission spectra in aqueous solution and on Sephadex DEAE A-25. No variation is observed in the peak wavelengths of the spectra in solid phase with respect to those ones in aqueous solution. The signal in the peaks is increased in about a factor of seven for a sample volume of 10 ml using 100 mg of Sephadex DEAE A-25.

The contour plot (Fig. 1), which shows the optimum wavelengths to measure the fluorescence signal, was obtained from the total luminescence spectrum. The maximum luminescence signal was found to be at 297 and 405 nm for excitation and emission wavelengths, respectively.

3.2. Effect of experimental variables

The influence of the variables potentially affecting the fluorescence intensity was studied in order to optimise the measurements conditions.

According to the SA molecule structure (which contains two ionisable groups that, after dissociation, yield two negative charges), various anion exchangers such as Sephadex DEAE A-25 and QAE A-25 were tested as solid supports at pH 7.0. It was found that the signal (and, consequently the sorption) was similar. DEAE A-25 was chosen because it offered the lowest background (11% lower than any of the other exchangers) so, this makes possible to measure the relative fluorescence intensity in a broader signal range.

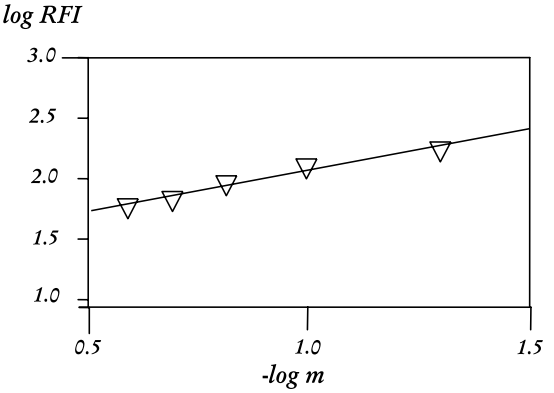

Fig. 2. Influence of amount of solid phase.

The influence of pH on the sorption and the fluorescence signal of SA on the solid support was studied by varying the pH value of a SA solution (10 ml) from 1.5 to 11.0 and equilibrating the solution with 100 mg of Sephadex DEAE A-25. The fluorescence signal measured on the solid phase increased as the pH value increased from 1.5 to 3.5. Above pH 3.5, the signal remained constant. So from pH 3.5, SA is completely under its salicylate anion form and the fluorescence signal is maximum. It seems to be that the presence of the anion exchanger makes the SA pK_a value (2.97 in aqueous solution) to lower due to the separation of anion salicylate from the solution and sorption on it, so displacing the dissociation equilibrium of SA towards the anionic form. This result is similar to that one described in other papers [24]. So pH 7.0 was selected as working pH.

One millilitre of a $H_2PO_4^-/HPO_4^{2-}$ buffer solution of $c_T = 0.1$ M at pH 7.0 was used to get the working pH. Higher volumes of buffer solution produced a significant decrease in the analytical signal due to the competition of the anionic buffer species for the active sites of the resin (using 5 ml of buffer the signal was 35% lower than that with 1 ml).

The solid support amount was studied to determine the minimum one offers a maximum fluorescence and, at the same time, it does not increase the stirring time. It was observed that the

Table 1
Analytical parameters

Analytical parameters	Sample volumes (ml)	
	10	25
Linear dynamic range (μg l^{-1})	10–200	5–100
Calibration graph		
Intercept	10.10 (0.65)[a]	5.0 (2.1)[a]
Slope (l μg^{-1})	2.90 (0.03)[b]	5.85 (2.18)[b]
Correlation coefficient (r)	0.998	0.998
Detection limit (μg l^{-1})[c]	2.8	1.1
Quantification limit (μg l^{-1})[d]	9.4	3.7
R.S.D (% $n=10$)	3.14	1.62

[a] Relative standard deviation (%) of the intercepts.
[b] Relative standard deviation (%) of the slopes.
[c] Criterion 3σ.
[d] Criterion 10σ.

fluorescence signal decreased as the resin amount increased. This is a consequence of the analyte dilution on the solid phase. It can be explained from the Eq. (1) that gives us the fluorescence signal as a function of the concentration of analyte in the initial solution c_0, and the volume, V of equilibrated solution and the solid phase amount, m.

$$I_f = \phi_f I_0 \varepsilon b \frac{c_0 V}{m(1 + V/(mD))} \quad (1)$$

where ϕ_f, quantum efficiency; I_0, radiant intensity of the excitation light; ε, molar absorptivity of the fluorophor (l mol^{-1} cm^{-1}); b, optical path length (cm); D, distribution coefficient (ml g^{-1}).

Usually, the fraction V/mD can be neglected against 1 and then I_f becomes inversely proportional to the amount of solid, m. It can be seen in Fig. 2 (graphical representation of log RFI against $-\log m$ is a straight line with slope = 0.70) the relation of inverse proportionality between the signal of fluorescence and the resin amount, as above explained.

So, the resin amount has to be minimum, which is restricted by the necessary amount to fill the cell. Finally, the working solid support amount was 100 mg.

On the other hand, from the intercept value obtained (1.47) and the experimental values used for $V = 10$ ml and $c_0 = 10^{-4}$ g l^{-1}, it could be evaluated the value of the constant $K = \phi_f I_0 \varepsilon b$ in Eq. (2):

$$I_f = \frac{KV}{m} c_0 \quad (2)$$

Taking into account that intercept = $\log(KVc_0)$, it was obtained a value of $K = 2.95 \times 10^7$.

From the slope values of the calibration graphics, KV/m (l μg^{-1}), in Table 2, it can be evaluated the experimental value of K. For $m =$ 0.100 g and using $V = 10$ and 25 ml, it was obtained a mean value of $K = 2.62 \times 10^7$. As can be seen, it is in good agreement with the value previously obtained Table 1.

The stirring time necessary for maximum fluorescence intensity development was 5 min for the two sample volumes assayed (10 and 25 ml). So, the results obtained show that the kinetics of the sorption process of the analyte on the resin is rapid. The centrifugation of the cell (containing the resin beads) before measuring did not increase

Table 2
Effect of foreign species

Foreign species	Tolerated interferent/analyte (w/w) ratio
Codeine, paracetamol	5
Manithol	2
Eucalipthol	1
Ascorbic acid, caffeine	0.25

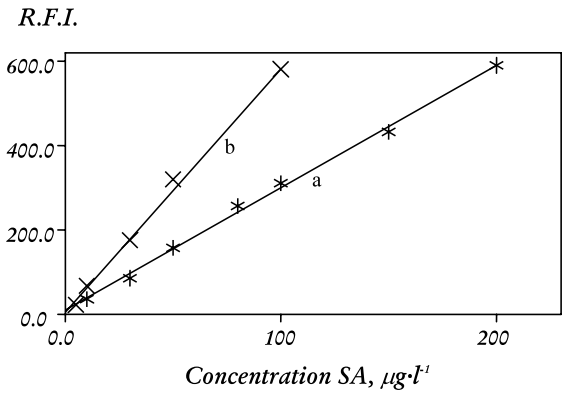

Fig. 3. Calibration lines for 10 (a) and 25 ml (b) sample volume.

Table 3
Analytical applications

Sample	Other species (mg)	ASA labelled (mg)	According to the method (mg)[a]	Added concentration ($\mu g\ l^{-1}$)	Mean recovery concentration ($\mu g\ l^{-1}$)	Recovery mean $\pm t\sigma_m$ (%)[a]
Couldina (Alter, Ltd.)	Chlorphenamine maleate(2), phenilephrine hydrochloride(7.5), manithol(200)	500	483 ± 7	50	49	98 ± 4
				100	98	98 ± 3
Dolvirán (Bayer, Ltd.)	Codeine phosphate(9.6), caffeine(50)	400	386 ± 4	50	49	98 ± 5
				100	98	98 ± 5
Veganín (Parke-Davis, Ltd.)	Codeine phosphate(10), paracetamol(250)	250	248 ± 6	50	48	98 ± 6
				100	99	99 ± 4
Fiorinal (Sandoz-Pharma, Ltd.)	Paracetamol(300), caffeine(40)	200	191 ± 3	50	49	98 ± 6
				100	99	99 ± 4

[a] Mean of three determinations.

the signal value. Nevertheless, the reproducibility was improved from 5.2% (without centrifuging) to 3.3% (for the 10 ml sample procedure) when the cell was centrifuged at 2000 rpm for 2 min.

The dependence of the fluorescence intensity of the analyte on the temperature was found to be critical as usual: increasing temperatures in the range 0–70 °C, the fluorescence intensity fell strongly, so, we chose a working temperature of 20.0 ± 0.1 °C using a thermostat. The fluorescence intensity was constant for, at least, 40 min and no photodecomposition was observed in this time.

The distribution coefficient has been evaluated. Buffer solution and 100 mg of Sephadex DEAE A-25 resin were added to an aqueous solution containing 3.62×10^{-4} mmol of SA and the solution (10 ml) was stirred for 5 min. The equilibrated solution was separated from the resin. Afterwards, the solution was treated in the same way with a further batch of resin (100 mg) in order to determine the concentration of SA left in the previously equilibrated solution as described under Section 2.5. The distribution ratio D (μmol of SA sorbed per g of resin per μmol of SA per ml of solution) was calculated from the initial and equilibrium concentration in the solution. An average value of $D = (1500 \pm 50)$ ml g^{-1} was obtained from five replicated experiments.

3.3. Analytical parameters

In the above established conditions, the fluorescence intensity of SA was found to be linearly related to its concentration in the range 10–200 and 5–100 ng ml^{-1} for 10 and 25 ml of sample volumes, respectively. Table 2 shows the analytical figures of merit of the proposed procedure. As can be seen, sensitivity (expressed as the slope of the calibration graph) increases as the sample volume increases. This is due to the preconcentration of an increasing amount of analyte on the same amount of solid phase as the sample solution volume used is increased. This increase can be seen in Fig. 3.

It can be seen a good agreement between the empirical slope ratio value, $s_{25}/s_{10} = 2.01$, and the predicted slope ratio value from the theoretical Eq. (3):

$$\frac{s_{v_1}}{s_{v_2}} = \frac{V_1}{V_2}\frac{(mD + V_2)}{(mD + V_1)} \quad (3)$$

which give us $s_{25}/s_{10} = 2.29$.

The reproducibility was established for ten independent analyses of solutions containing 100 μg l^{-1} of analyte for 10 and 25 ml. The detection limit was estimated by using the 3σ-criterion [31] and the quantification limit by using the 10σ-criterion [32]. As can be seen, the procedure shows a detection limit better than other spectroscopic procedures described in the literature [1,9,37,38]. Also sensitivity is higher than that of spectroscopic and chromatographic procedures. The procedure proposed compare very favourably with conventional spectroscopic procedures in terms of sensitivity, selectivity and simplicity. Although chromatographic procedures [6–8,33–36] are more suitable when several other active principles accompanying to (A)SA have to be also analysed, the solid phase fluorimetric procedure developed is quicker and inexpensive and can be applied to pharmaceutical routine control analysis of (A)SA.

3.4. Effect of foreign ions

In Table 2 the effect of various potential interferent species, commonly found in pharmaceutical preparations together with SA, on the determination of 100 ng ml^{-1} is shown. This effect was investigated by adding a known amount of the tested species to the SA solution. Tolerance level is defined as the foreign species concentration that produces not more than ±5% spectrofluorimetric error in the recovery of SA.

It can be seen that the method proposed shows a good level of tolerance to the tested species, being appropriate for pharmaceuticals analysis. The more important interference was found to be Caffeine and Ascorbic Acid. Nevertheless, the amount of Caffeine present in pharmaceuticals preparations is minor than the level of tolerance.

3.5. Applications

The proposed procedure was applied to the determination of SA in pharmaceutical preparations. Usually SA is found as ASA, so a previous alkaline hydrolysis was performed as described above (Section 2.4). To validate the procedure, a recovery study was performed by adding two levels of known concentrations of SA (50 and 100 ng ml^{-1}) to the assayed drugs. Table 3 shows good mean percents of recovery, so testifying the accuracy of the method.

4. Conclusions

A single and straightforward solid-phase spectrofluorimetric procedure for the determination of SA and indirect determination of ASA has been developed. The procedure does not use any derivative reagent, so it is quicker and more inexpensive than chromatographic methods [6–8,33–36]. The sorption of the analyte on the solid phase from the solution provides, both a significant improvement in selectivity (because the species excluded from the resin can not interfere) and a high increase in sensitivity. So, a detection limit as low as 1.1 μg l^{-1} can be achieved, much better than those provided by spectrophotometric [1,37] or even by the conventional spectrofluorimetric methods in solution [10,38]. In addition, good reproducibility is obtained. This method contributes to expand the scarce applications of Solid Phase Spectrofluorimetry in batch mode to the pharmaceutical analysis field, and it has the advantage of being easy to use, so it can be applied in any laboratory.

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